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利用拮抗菌 *Trichoderma koningii* 對紅豆根腐病之生物防治

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摘　要

劉顯達 1991 利用拮抗菌 ***Trichoderma koningii*** 對紅豆根腐病之生物防治　植保會刊 33:63～71

紅豆根腐病爲土壤病原菌 *Rhizoctonia solani* AG-4 引起之病害，防治不易。由土壤中篩選出强拮抗性之眞菌 *Trichoderma koningii* 對本病菌具重寄生作用，會纏繞及分解 *R. solani* 菌絲。利用化學誘變劑處理拮抗菌，得到一個耐藥性菌系 T12-R33-D25，該菌可耐 Benlate 至 1000 ppm (a.i.) 且於植物之根部拓殖能力顯著增加。利用稻殼米糠培養拮抗菌經十天後每克產孢量達 10^9 可作爲田間防治應用之量產基質。於紅豆植穴中添加約 1 g 稻殼米糠拮抗菌培養物或以拮抗菌孢子懸浮液包覆紅豆種子上，顯著增加紅豆之健康植株數約 56 至 14%，每株產量也增加。如同時添加其他機質，根腐病之發病率也會降低，但和單獨添加拮抗菌處理並無差異。拮抗菌懸浮液處理種子之技術較爲簡易可用來大面積田間推廣紅豆根腐病之生物防治。

（關鍵字：生物防治 *Rhizoctonia solani*、*Trichoderma koningii*、紅豆）

紅豆根腐病

紅豆爲高屏地區之重要冬作，年產總値近十億新台幣，然紅豆生育全期均會受到土壤病原菌 *Rhizoctonia solani* AG-4 爲害，影響產量至鉅。*R. solani* 爲土壤病原菌，於土壤中殘存期長，腐生能力强，寄主範圍廣，防治困難，本菌爲害紅豆，造成紅豆初期種子腐爛，或萌發出土前及出土後之猝倒病（pre or post-emergency damping-off），或造成幼菌或成株之根腐病（root rot），一般以藥劑防治難達到經濟性之防治目標，且易造成環境污染，故以生物防治技術作爲防治策略方爲適切可行之途徑[1,4,10,15]。

拮抗菌與生物防治

以 *Trichoderma* spp. 作爲拮抗微生物來防治植物病害之研究，近年來頗受植物病理學者重視，亦發表了不少報告。例如土壤病害，植物地上部器官及儲藏期病害之生物防治。然只限於實驗室、溫室或小型田間試驗能成功，眞正推廣作爲田間防治者，則尙待努力與突破。植物病害之發生與流行和環境、時間關係密切，某些地區某些病害會特別嚴重，反之，某些地區之某特定病害卻很少，這其中可能意謂者生態系中自然生物防治之存在，植物病理學者若要從其中去探索何種因素控制著這生物防治，實需投注更多心血，從各種角度去研究，並加

以評估。本人曾報告 *R. solani* 之抑病土（suppressive soil）之主因乃由於 *Trichoderma* sp. 族群密度之增加[12]。Hadar, Henis 及 Chet 氏等報告以 *Trichodermar* spp. 防治 *R. solani* 造成之蕃茄猝倒病、康乃馨根腐病均有優良之效果[4,6,7]，Elad 氏更指出 *Trichoderma hazianum* 對菜豆（*Phaseolus vulgaris*）之 *R. solani* 及 *Sclerotium rolfsii* 二種病菌亦有生物防治之功效[5]。此外眞菌對 *R. solani* 具有拮抗性之報告有 *Aspergillus niger*、*A. flavus*、*A. candidus*、*Gliocladium roseum*、*Trichoderma viride*、*Corticium* sp.、*Chaetomium cupreum*、*Laetisaria aivalis*、*Gliocladium virens*、*Verticillium biguttatum*. 拮抗細菌者有 *Bacillus subtilis*、*B. uniflagellatus*、*Pseudomonas fluorescens* 等。此等拮抗生物之作用大部分均爲於實驗中或溫室盆栽試驗表現出其生物防治效果，僅有少數如 *T. harzianum* coating 在鳶尾之球莖，*T. hamatum* coating 在棉花種子或以 *Trichoderma harzianum* 混入消毒之土壤，種植蕃茄等在田間獲致對 *R. solani* 有生物防治作用。生物防治於田間能否獲得成功，田間環境影響甚鉅；拮抗菌族群之建立、繁殖、殘存之生態條件，*R. solani* 之菌絲，分佈及感染源形態，密度殘存等在在均會影響生物防治之運作。

Beagle-Ristaino 以 *Trichoderma viride* [T-1-R9] 及 *Gliocladium virens* [G1-21] 作拮抗菌成功的於溫室及田間防治馬鈴薯菌核病，發病率減少 54～89%，而其所使用 T-1-R9 菌株對 benomyl 具抗藥性，爲成功之重要因素。本試驗所篩選之拮抗菌 T12、TC11、TVCN1、T2、TD 及 T33 對 benomyl 均無抗性，於 1 ppm 濃度下生長完全受抑制。因現行之苗圃或本田期，農民大量施用 benomyl 來防治病害，爲期添加之拮抗菌能發揮生物防治作用，篩選抗藥性之拮抗菌爲重要課題[2,13]。

篩選強拮抗性之優良菌株

由紅豆田間土壤及紅豆植株包括根系部位之組織，利用土壤稀釋法，組織表面培養法及尼龍網誘菌法等[11,12]分離到近百種菌株（細菌與眞菌），利用對峙培養及玻璃紙抗生法先行測試各菌株對 *R. solani* AG-4 之拮抗能力，得到拮抗眞菌 *Trichoderma koningii* T-12、*T. harzianum* TC-11 及拮抗細菌 A003、A020、A036、A045、A047 及 A090 等菌株。其中 T-12 及 TC-11 對 *R. solani* 之重寄生現象尤爲明顯，會纏繞及分解該菌絲，在對峙培養時，即使兩種菌絲尙未接觸，*R. solani* 菌絲前端細胞就表現出不正常的生長現象，如細胞縊縮、細胞質顆粒化等。*Trichoderma* 和 *R. solani* 在土壤中彼此間族群密度消長則利用選擇性培養基配合土壤孔土片法測定之[5,8,9,12]。

拮抗菌抗藥性之誘變

由於 *Trichoderma* 拮抗菌對於 benomyl 藥劑非常敏感，1～10 ppm 即會明顯抑制 *Trichoderma* 生長，故如需施用該拮抗菌於田間作爲生物防治，能順利建立族群，於田間繁殖殘存具有耐藥性或抗藥性是必須的。將 T-12 及 TC-11 菌株利用 uv 照射處理或是以化學誘變劑 DES、EMS 及 N_aNO_2 處理得到一些菌株（表一），再一步測試其耐藥性時，發現 T12-R33-D25 菌株對 benomyl 之抗藥性最好（表二），而對 PCNB 及 Neo Asozin 之抗藥性則和親本型 T-12 則無差異（圖一），而此抗藥性菌株 T12-R33-D25 對 *R. solani* 之重寄生作用或抗生作用並無衰退或減少（表三）。本試驗利用物理及化學誘變法處理拮抗菌 *Trichoderma koningii* 及 *T. harzianum*，成功地由 DES 處理得到 T12-R33-D25 菌株可容忍 Benlate 1000 ppm，比目前發表之 T1-R9 及 T95-GA[3,13] 耐藥性還高，且產孢能力十分正常，T95-GA 只會在含 benomyl 之培養基才會正常產孢。

拮抗菌於紅豆根部拓殖現象

一般而言，眞菌比細菌於植物根部表面拓殖能力（root colonization）較差，若應用拮抗菌作爲植物根部病害之生物防治，該拮抗菌之根部拓殖能力影響其防治效果很大。利用 Randhawa 氏之幼苗測試法[14]及鋁箔管法[3]二種方法來檢定拮抗菌根部拓殖能力時，發現抗藥性拮抗菌之拓殖能力大幅提高（表四），此現象之發現和 Baker 氏之報告不謀而合，至於

爲何抗藥性之拮抗菌會有此特異性，其作用機制爲何現仍探討中。

紅豆根腐病生物防治效果溫室測試

溫室盆栽試驗中，發現添加 T12 於病土中，紅豆根腐病之發病率由 47% 降爲 22%，若添加 T12-R33-D25 於病土中則更降爲 9%，若僅添加 chitin（0.1%），或 CMC（0.1%），或 C_aSO_4（0.1%），或稻殼（0.1%），或蝦殼粉（0.05%）於病土中，則只有 CMC 添加處理之發病率稍爲降低，其他則無差異（表五）。由此顯示拮抗菌添加處理之生物防治效果十分明顯，特別是 T12-R33-D25 處理發病率尤低，其效果應和其根部拓殖能力有關。而病土中添加 CMC 也可以降低發病率，可能和促進土壤中自然存在微生物繁殖有關，特別是易於利用及分解纖維素之微生物。

表一、利用物理及化學誘變處理拮抗菌 *Trichoderma* spp. 所得到新菌株之數目

Table 1. The number of biotypes of antagonistic *Trichoderma* spp. obtained from physical and chemical mutation treatments

		Biotype			
		UV[1]	DES[2]	EMS[2]	$NaNo_2$[2]
Trichoderma harzianum	TC11	15	12	24	6
Trichoderma koningii	T12	17	46	73	11

1) Conidial suspensions were irradiated under 255 nm wavelength at the density of 190 W/cm^2 for 2-4 hr.

2) Diethyl sulfate (DES), ethyl methanesulfonate (EMS) and sodium nitrite ($NaNO_2$) were applied as chemical mutagens.

表二、拮抗菌 *Trichoderma* spp. 耐藥性菌株於含不同濃度 benomyl 培養基之生長菌落直徑

Table 2. Colony diameter[1] of mutants of antagonistic *Trichoderma* spp. grown on various concentrations of benomyl

Biotype[2]	Benomyl concentration (μg/ml)										
	0	1	10	20	50	100	200	400	600	800	1000
T12	90	7.3	7.2	–	–						
TC11	90	8.7	–	–							
TC11-R5	90	81	35	–							
T12-R33	90	90	63	37							
T12-R33-D19					89	63	32	30	27	26	–
T12-R33-D25					90	62	38	41	37	34	26
T12-R33-D31					90	54	31	30	29	26	–
T12-R33-D75					79	43	27	24	23	21	–
T95-GA					80	24	19	17	–		

1) Antagonistic fungi were cultured on PSA plate with various concentrations of benomyl, incubated at 28℃, and colony diameters measured in mm after 72 hr incubation.

2) T12 isolate is *T. koningii*, TC11 isolate is *T. harzianum* and T95-GA also *T. harzianum* (from R. Baker).

表三、*Trichoderma koningii* 抗 benomyl 突變菌株對 *Rhizoctonia solani* 之重寄生及抗生作用

Table 3. Mycoparasitism and antibiosis assay of benomyl resistant mutants of *Trichoderma koningii* against *Rhizoctonia solani*

Biotype isolate	Hyperparasite[1)]		Antibiosis[2)]
	Hyphae coiling	Hyphae lysis	Colony diam. (mm)
T12	+	+	17.5
T95-GA	+	+	44.9
T12-R33	+	+	18.4
T12-R33-D19	+	++	23.5
T12-R33-D25	+	++	15.5
T12-R33-D31	+	++	20.3
T12-R33-D36	+	+	20.7
T12-R33-D37	++	+	20.3
T12-R33-D75	+	+	15.6
T12-R33-D83	++	++	14.2

1) ++, + means degrees for hyphae coiling by *Trichoderma* and hyphae lysis of *Rhizoctonia* assayed by dual culture method.

2) Assayed by cellophane membrane method.

表四、拮抗真菌及細菌於蘿蔔根部拓殖情況

Table 4. Rhizosphere colonization of antagonistic fungi and bacteria on the radish roots

Seed coating antagonist	Root tip colonized[1)] by mutant	Depth of root colonized[2)] by antagonist (cm)
A003	+	2
A020	+	8
A036	+	8
A045	−	2
A047	−	1
A090	−	2
TC11	−	2
T12	−	3
T12-R33	+	8
T12-R33-D25	+	8
T95-GA	+	8

1) Assayed by Randhawa's seedling bioassay method; + = colonization, − = no colonization.

2) Assayed by Chao's aluminum foil tube method with sterilized sandy loam soil.

表五、土壤中添加物及拮抗菌對紅豆根腐病之生物防治效果

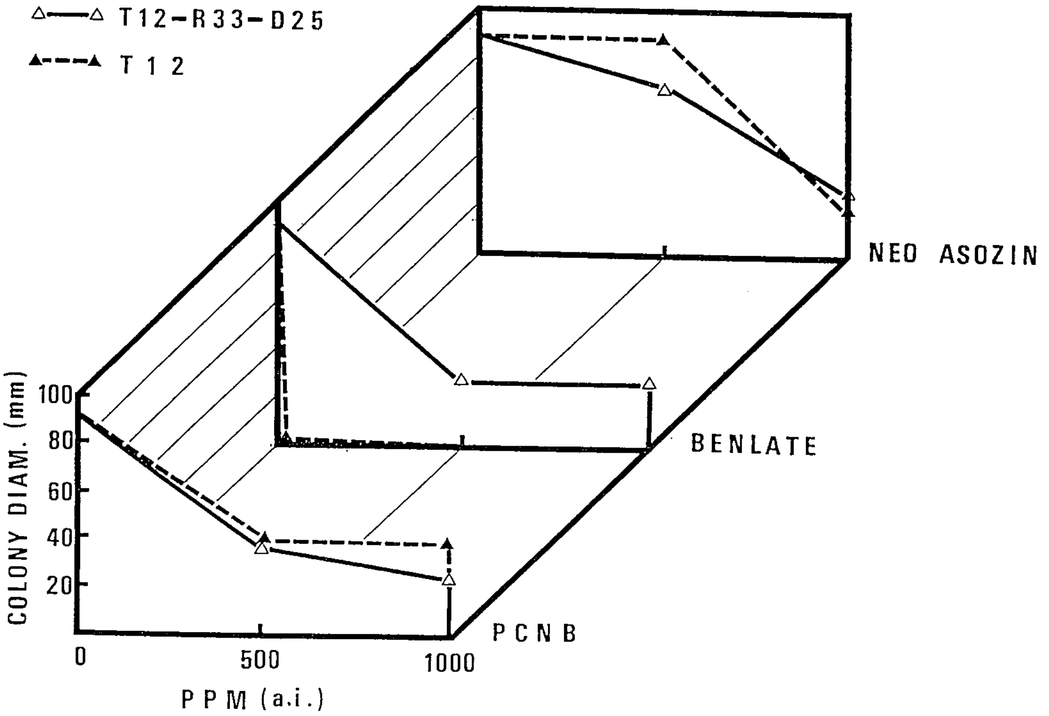
Table 5. Biocontrol of adzuki bean root rot by soil amendment with additives and antagonists

Amendment treatment	Disease incidence (%)[1]			
	Re[2]	Re + T12[3]	Re + D25[3]	Control
Chitin 0.1%	37 ab	17 ab	7 b	15 a
CMC 0.1%	29 b	18 bc	9 ab	8 b
$CaSO_4$ 0.1%	44 a	28 a	14 a	9 b
Rice husk 0.1%	44 a	28 a	12 a	12 a
Shrimp shell 0.05%	32 ab	20 b	15 a	11 ab
Control	47 a	22 ab	9 a	5 b

1) Disease severity was the average of five replicates each treatment, and each replicate had 10 pots planting 10 seeds in each. Data in each column followed by different letters were significantly different according to Duncan's multiple range test (p = 0.05).

2) Soil infested with CPS inoculum of *Rhizoctonia solani* Re isolate at the density of 5 mg/g soil.

3) Soil also amended with the conidia of *Trichoderma koningii* T12 or T12-R33-D25 harvested from rice husk media culture at the density of 10^7 conidia/g soil.



圖一、拮抗菌 *Trichoderma koningii* T12 菌株及耐藥性突變菌株 T12-R33-D25 對 PCNB, Benlate 及 Neo Asozin 三種農藥之殺菌反應

Fig 1. The fungicidal response of antagonistic fungi T12 (*Trichoderma koningii*) and T12-R33-D25, a benomyl resistant mutant, to PCNB, Benlate and Neo Asozin fungicides.

拮抗菌量產基質及方法

Papavizas 指出添加 *Trichoderma* 於土壤中作爲生物防治因子時，以厚膜孢子發芽較優，殘存期也長[13]故探討適當且價值低廉之培養基質，促進拮抗菌能同時產生大量之厚膜孢子及分生孢子，且培養方法簡易能達到量產目的。

稻殼和米糠混合製成之培養基（含水量 50% W/W），以2：1 (W/W)之比例，T12 分生孢子濃度最高可達 2.5×10^9 conidia/g，T12-R33-D25於4：1或2：1之混合比例中，分生孢子濃度無顯著區別，均約可達 10^8～10^9 condidia/g，而 T12-R33-D25 產生厚膜孢子之能力於4：1或2：1、1：4均較T12爲少，於2：1比例之培養基厚膜孢子可達 $5 \sim 6 \times 10^7$ chlamydospores/g（圖二），利用酒糟（米糟），糖蜜及酵母作液體培養對 T12及T12-R33-D25之產孢量無甚差異，唯於米糟中T12-R33-D25分生孢子量幾乎達到 10^8 conidia /g，但仍比稻殼米糠培養基產孢量差（圖三）。

目前量產之方法即以耐熱之 PP 袋裝盛稻殼米糠培養基經高壓滅菌後接種拮抗菌孢子懸浮液，於 28℃培養 14 天後備用，另外以其他基質，經醱酵法，或半消毒法培養亦有良好成效，培養物作爲粒劑、液劑及可濕性粉劑，其有效性及儲藏期正探討中。

紅豆根腐病田間生物防治試驗及推廣

民間 76 年冬作，於內埔、潮洲二地各一甲地進行試驗，土壤中有添加病原菌處理，拮抗菌以植穴添加及種子處理二種施用方式，結果發現以拮抗菌添加處理效果最好，健株較未添加拮抗菌處理曾加 55.9%，產量增加 28.4%（表六），種子處理效果未盡理想。民國 77 年及 78 年冬作再進行田間試驗，唯不再添加病原菌作爲接種源，種子處理之孢子懸浮液也改進爲添加 2% CMC及 3% arabic gum，孢子濃度提高爲 10^9孢子 /ml，每公頃種子量 70～75 kg 約需 2.5 l孢子懸浮液拌種處理後涼乾即可播種。結果發現拮抗菌添加及拌種二處理均顯著增加紅豆產量，每公頃約 1200公斤至500公斤（表七），因利用拮抗菌拌種成本低廉，施用簡易，現正配合紅豆高雄五號品種之推廣實施拮抗菌拌種推廣試驗作不同區域性之觀察比較。

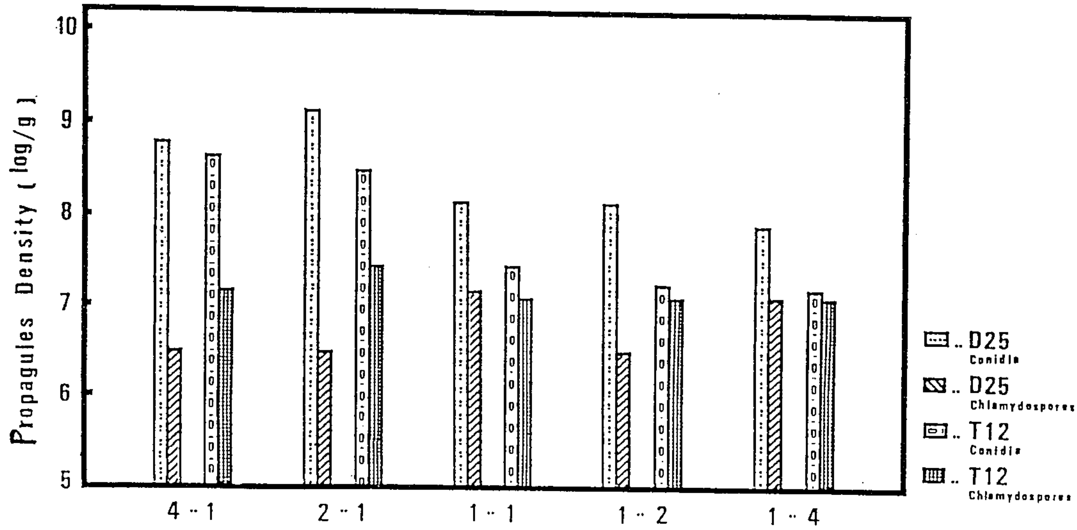
表六、抗藥性拮抗菌 *Trichoderma koningii* D-25 對紅豆根腐病生物防治效果

Table 6. Biocontrol effect of benomyl resistant antagonist *Trichoderma koningii* D-25 against root rot disease of adzuki-bean

Treatment	Plant stand		Yield	
	No/0.1 ha	%	kg/0.1 ha	%
Noninoculated Ck	22019	100 b	169.09	100 b
Inoculated with				
R. solani				
D-25 coating	19455	88.4 ab	156.17	92.4 ab
D-25 amending	28760	130.6 c	193.58	114.5 c
without D-25	16441	74.7 a	145.58	86.1 a

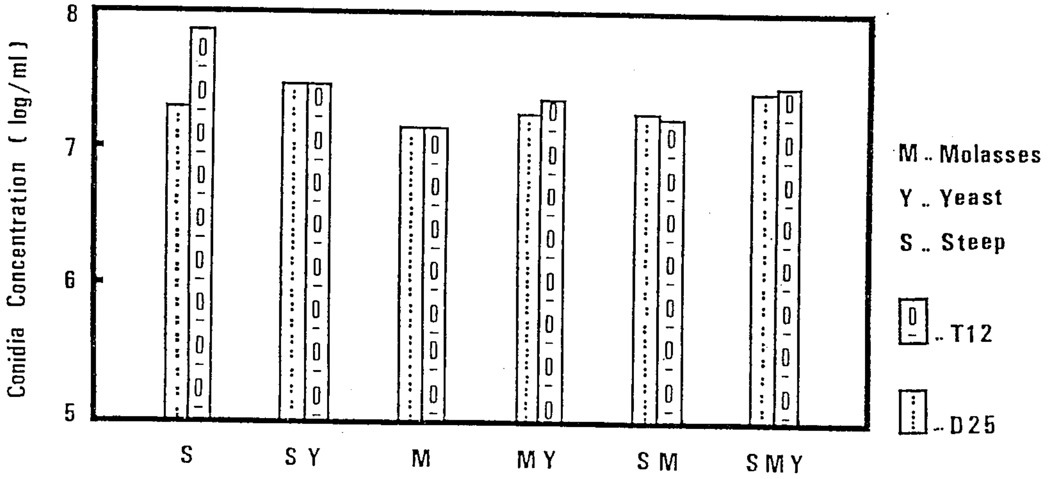
a. CPS inocula of *Rhizoctonia solani* were added to sowing hole at ca. 5 mg per hole. In D-25 amending treatment, 1 g of rice husk cultured biomass with conidia 10^9 per g was amended into hole at the same time. In seed coating treatment, adzuki-beans were dipped into conidial suspension (10^9 conidia per ml) with 1 % CMC for 5 mins and air dry.

b. Values in each column followed by the same letter are not significantly different ($p = 0.05$) according to Duncan's multiple range test.



圖二、拮抗菌 *Trichoderma koningii* T12及耐藥性菌株 T12-R33-D25於不同比例之稻殼米糠培養基（28℃，14天）產生分生孢子及厚膜孢子之情形

Fig 2. The sporulation of conidia and chlamydospores of *Trichoderma koningii* T12 and benomyl resistant isolate, T12-R33-D25 cultured on various formulating rice husk-bran media (ratio of rice husk to bran) at 28℃ for 14 days incubation



圖三、拮抗菌 *Trichoderma koningii* T12及耐藥性菌株 T12-R33-D25於米糟、糖蜜、酵母培養基之產孢量

Fig 3. The sporulation of conidia and chlamydospores of *Trichoderma koningii* T12 and benomyl resistant isolate, T12-R33-D25 cultured on rice steep, molasse of yeast extract at 28℃ for 14 days incubation

表七、抗藥性拮抗菌 *Trichoderma koningii* D-25 對紅豆根腐病田間生物防治效果

Table 7. Biocontrol effect of *Trichoderma koningii* D-25 on adzuki bean root rot disease, caused by *Rhizoctonia solani*

Treatment[2]	Dried wt. (g/plant)				Yield (g/plant)				Yield (kg/ha)			
	1[1]	2[1]	3[1]	av.	1	2	3	av.	1	2	3	av.
Soil amending	44.6	52.8	74.4	57.3 b[3]	7.8	9.3	11.8	9.6 b	4026	4135	4517	4226 c
Seed coating	58.1	50.7	60.8	56.5 b	9.2	8.5	9.9	9.2 b	3243	3158	4123	3508 b
CK	34.7	28.2	43.5	35.5 a	5.8	4.6	7.3	5.9 a	3141	2783	3127	3017 a

1) Plot 1 and 2 were 1 hectare each, plot 3 was 2 hectares and each plot had five replicates designed randomly for field trial.

2) About 5 g of antagonist biomass were put into sawing holes in soil amending treatment and conidial suspension at density of 10^8 conidia per ml with 2% carboxymethyl cellulose and 3% arabic gum was used in seed coating treatment.

3) The data in each column following the same letter are not significantly different according to Duncan's Multiple Range Test (p = 0.05).

引用文獻

1. Baker, R., and Martinson, C. A. 1970. Epidemiology of disease caused by *Rhizoctonia solani, in Rhizoctonia solani,* Biology and Pathology (J. R. Parmeter, Jr., ed.), p.172-188. Univ. Calif. Press, Berkeley.
2. Beagle-Ristaino, J. E., and Papavizas, G. C. 1985. Biological control of *Rhizoctonia* stem canker and black scurf of potato. Phytopathology 75:560-564.
3. Chao, W. L., Nelson. E. B., Harman, G. E., and Hoch, H. C. 1986. Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology 76:60-65.
4. Chet, I., and Baker, R. 1981. Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani.* Phytopathology 71: 286-290.
5. Elad, Y., Chet, I., and Henis, Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica 9:59-67.
6. Hadar, Y., Chet, I., and Henis, Y. 1979. Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum.* Phytopathology 69: 64-68.
7. Henis, Y., Chaffar, A., and Baker, R. 1978. Integrated control of *Rhizoctonia solani* damping-off of radish: effect of successive plantings. PCNB, and *Trichoderma harzianum* on pathogen and disease. Phytopathology 68:900-907.
8. Henis, Y., Shaffar, A., Baker, R., and Gillespie, S. L. 1978. A new pellet soil sampler and its use for the study of population dynamics of *Rhizoctonia solani.* in soil. Phytopathology 68:371-376.
9. Ko, W., and Hora, F. K. 1971. A selective medium form the quantitative determination of *Rhizoctonia solani.* in soil. Phyto-

pathology 61:707-710.

10. Lin, S. C., Wang, M. H., and Liu, S. D. 1986. Integrated control of stem rot disease of chrysanthemum--I. The biocontrol effect by antagonists and fungicide tolerance assay. Bulletin. Natl. Pingtung Inst. Agric. 27:60-68.
11. Liu, S. D., 1982. Systematic isolation and screening antagonists suppressive to *Rhizoctonia solani.* Natl, Sci. Counc. Monthly, R.O.C. 10:348-355.
12. Liu, S. D., and Baker, R. 1980. Mechanism of biological control in soil suppressive to *Rhizoctonia solani.* Phytopathology 70:404-412.
13. Papavizas, G. C., and Lewis, J. A. 1983. Physiological and biocontrol characteristics of stable mutants of *Rhizoctonia solani.* resistant to MBC fungicides. Phytopathology 73:407-411.
14. Randhawa, P. S., and Schaad, N. W. 1985. A seedling bioassay chamber for determining bacterial colonization and antagonism on plant roots. Phytopathology 75: 254-259.
15. Wu, W. S., Liu, S. D., Chang, Y. C., and Tschen, J. 1986. Hyperparasitic relationships between antagonists and *Rhizoctonia solani.* Plant Prot. Bull. (Taiwan. R.O.C.) 28:91-100.

ABSTRACT

Liu, S. D., 1991. **Biological control of adzuki-bean root rot disease caused by *Rhizoctonia solani.*** Plant Prot. Bull. 33:63-71. (Department of Plant Protection, National Pingtung Institute of Agriculture, Pingtung, Taiwan, Rep. of China)

The root rot disease of adzuki-bean (red bean, *Phaseolus angularis*) caused by *Rhizoctonia solani.* is an important and destructive adzuki-bean disease in Taiwan. Conventional chemical control is impractical. Biological control or *R. solani* with antagonist(s) may be and alternative for control of the disease induced by this soil-borne pathogen. By in vitro dual culture and antibiosis bioassay, two antagonistic fungi, *Trichoderma harzianum* (TC11) and *T. koningii* (T12) were shown to be strongly antagonistic to *R. solani* from sreening tests. To improve the biocontrol efficacy and surcival in field where a number of fungicides were constantly sprayed, TC11 and T12 were induced to be benomyl resistant. A new strain (T12-R33-D25) obtained was resistant to 1000 $\mu g/ml$ benomly, without decreasing mycoparasitic, lytic ability or antibiosis against *R. solani* compared with the parent strain. A rhizosphere competent phenomenon and growth promotion effect were observed in seed coating or soil amendment with T12-R33-D25. With soil amending of culture biomass ca. 1 g into sawing hole or seed coating with conidial suspension, the healthy plants and yields of adzuki-bean significantly increased compared with non-biocontrol treatment in field trials at Pingtung and Kaohsiung from 1986 to 1990. *R. Solani* was successfully controlled by these two delivery systems with benomyl resistant antagonistic *T. koningii,* but application by seed coating consmued less labor hours and culture produce of antagonist is now under extension to the bean grower.

(Key words: Biocontrol, *Rhizoctonia solani, Trichoderma koningii,* adzuki-bean)